POLYMER GEL CONTACT MASKS AND METHODS AND MOLDS FOR MAKING SAME

FIELD OF THE INVENTION

The present invention relates to polymer gel patterning elements such as contact masks and diffusion masks and molding methods for making these patterning elements including complementary molding techniques.

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BACKGROUND OF THE INVENTION

In the fields of drug development, cellular biology, tissue engineering and physiology, there is now a need for devices with features on the size scale of individual cells. In particular, devices for controlling the spatial arrangement of assay materials in a format of arrays are 10 important in these fields. In drug development, arrays are used to increase throughput and decrease the labor involved in screening compounds. High throughput screening requires parallel handling of chemical compounds, biochemical reagents, biological targets and other reagents, collectively referred to herein as biological materials.

Advances in high throughput screening depend upon automation, miniaturization and 15 improved detection technology. Examples of biological assays that have been automated include ligand receptor binding assays, scintillation proximity assays, enzyme assays, enzyme-linked immunosorbent assays (ELISA), reporter gene assays, cell proliferation and toxicity assays, endotoxin assays and agglutination assays. The present invention relates most closely to

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miniaturization.

Younan Xia and George M. Whitesides, "Soft Lithography" Angew. Chem. Ins. Ed. 37, 550-575 (1998) describes methods for patterning a variety of materials on surfaces using pattern-transfer elements made of polydimethylsiloxane ("PDMS"). In one extension of soft lithography, a PDMS contact mask is used to shield a substrate in all areas except where there are holes through the contact mask. Material is then passed through the holes and onto the exposed portions of the substrate.

In at least one significant way, PDMS pattern transfer elements are better adapted to patterning of inorganic materials, such as electroluminescent compounds for optical displays, such as is described in Duffy, et. al *Adv. Material* 1999, *11*, 546-552, than they are for patterning biological materials, because PDMS is cytophilic. Consequently, when patterning cells using a PDMS patterning element, a large proportion of the cells intended for deposition onto the substrate are diverted because they adhere to the patterning element.

With the development of high throughput screening assay chips for use in drug discovery and medical research there is now a need for patterning elements that are non-cytophilic to complement, and in some instances to replace, PDMS elements used in these applications. The present invention meets this need by providing patterning elements made of materials with low cytophilicity and good biocompatibility.

In addition, polymers that form gels with water have properties similar to networks of proteins and polysaccharides that make up much of the extracellular material of the human body. This fact has spurred an interest in using hydrogels in medical implants. These materials may be well suited for creating an *in vitro* environment for the study of cells and other biological materials.

The handling of minute quantities of chemicals and biological materials as required for high throughput screening of drug candidates is a challenge currently facing the pharmaceutical industry. For instance, the lack of suitable liquid handling devices has been cited as a stumbling block to further screen miniaturization. Stylli, H; "An Integrated Approach to High Throughput

5   Screening" in *Handbook for the 1994 International Forum on Advances in Screening Technologies and Data Management* at p. 5. Methods have been developed to position such minute quantities in a small area using robotic equipment and ink jet delivery devices. Commonly-assigned co-pending Patent Applications Serial Nos. [atty. docket 11641/5 and 4] disclose alternative methods of positioning minute quantities of material in a small area on a

10   biological array that are compatible with and complementary to robotic methods. One such method involves placing a contact mask over a substrate to conceal a portion of the substrate and leave a plurality of discontinuous portions of the substrate exposed. Such a mask has a plurality of holes through it. Each of the holes, together with the portion of the substrate surface which it overlies, forms a cavity. Biological and chemical materials can be deposited into each of the

15   cavities individually using robotic equipment, or collectively by immersion in a solution, spraying, brushing or dropwise deposition using far less sophisticated and expensive equipment than is conventionally used to address individual elements of an array. PDMS has desirable adhesion, elasticity and strength properties and can be cast from a non-viscous precursor so that minute features like 50 μm holes are transferred from a mold master to the PDMS. PDMS,

20   however, is cytophilic, which is problematic for patterning cells, proteins and other biological materials in an array because these materials tend to adsorb onto the PDMS. In many applications for which bioarrays are suited, it is necessary to deposit material over the entire array or into a large group of adjacent cavities. Depositing material over a large contiguous portion of

an array has potential cost savings in fabricating and using the array because these steps can be conducted without expensive and time consuming robotic manipulations. However, when large areas of the array are addressed collectively, it is detrimental to the process if the material adheres to the top surface of the contact mask instead of depositing into a cavity. In addition, material adsorbed by the PDMS in one patterning step may interfere or react with material being patterned in a subsequent step. The present invention provides a solution to this problem by providing a non-cytophilic patterning element, such as a contact mask, that has the desired properties of adhesion and elasticity (akin to those of PDMS) and acceptable tensile strength for use in patterning of biological and chemical materials on microarrays.

SUMMARY OF THE INVENTION

In accordance with one embodiment of the present invention, a polymer gel patterning element for patterning biological materials is provided. In another embodiment, the present invention provides a polymer gel contact mask. Yet another embodiment of the present invention provides a contact mask comprising a polymer gel having at least one hole therethrough. Another embodiment of the present invention comprises a polymer gel contact mask formed by complementary molding.

The present invention also provides a mold for producing a hydrogel contact mask from a precursor composition, the mold comprising first and second half molds wherein the first half mold is made from an elastomer and deforms to accommodate dimensional changes in the precursor composition as it cures into a hydrogel. In another embodiment, the present invention includes a mold comprising a half mold produced by thermal imprinting with an elastomeric master. The present invention further provides a complementary mold comprising first and

second half molds each having molding surfaces, wherein the molding surfaces define a void when the first and second half molds are closed and wherein at least one of the first or second half molds is an elastomer. Yet another embodiment of the present invention includes a complementary mold comprising a PDMS half mold having a first molding surface, a release film adjacent to the first molding surface, and a rigid half mold having a second molding surface, wherein the first and second molding surfaces define a void.

The present invention further provides a method of complementary molding comprising the steps of providing a complementary mold including first and second half molds each having molding surfaces, wherein the molding surfaces define a void when the first and second half molds are closed and wherein at least one of the first or second half molds is an elastomer. Further wherein a polymerizable precursor is introduced, the half molds are closed, and the molding precursor is polymerized. Alternatively, the polymerizable precursor can be introduced into the void after the mold surfaces form the seal.

In another embodiment of the present invention, a method of complementary molding comprises the steps of providing a complementary mold including first and second half molds each having a molding surface, the molding surfaces defining a void there between, wherein the half molds close to form a void, and wherein at least one of the first or second components is an elastomer. The method further includes closing the half molds to form a void, introducing a polymerizable precursor into the void after the mold surfaces form the void, and polymerizing the molding precursor.

In yet another embodiment of the present invention, a method of continuous complementary molding comprises the steps of providing a first half mold including a plurality of first molding surfaces adapted and arranged for moving the molding surfaces from an

unprocessed position to a processed position, providing a second half mold having a second

molding surface, wherein the first and second molding surfaces form a void there between when

closed, applying polymerizable polymer precursor to one of the molding surfaces in the

unprocessed position, closing the half molds, polymerizing the polymer precursor, and moving

5   the molded polymer to the processed position.

Another embodiment of the present invention includes a method of thermal imprinting

comprising the steps of providing an elastomeric master having a first imprinting surface and a

elastomeric substrate having a second imprinting surface, introducing a film having a lower

glass transition temperature than the elastomeric master and the elastomeric substrate, between

10   the elastomeric master and the elastomeric substrate, heating the film to a temperature above the

glass transition temperature of the film, and cooling the film. Yet another embodiment of the

present invention includes a method of forming a rigid complementary half mold comprising the

steps of providing a first PDMS master having a first imprinting surface with surface features,

providing a second PDMS substrate having a second imprinting surface, providing a

15   polyethylene film between the first and second imprinting surfaces, applying pressure to the

layered structure, heating the layered structure above the glass transition temperature of the

polyethylene, and cooling the layered structure.

BRIEF DESCRIPTION OF THE DRAWINGS

20   The various features of the invention will be appreciated by simultaneous reference to the

description which follows and the accompanying drawings.

Fig. 1 illustrates a complementary molding process for forming an elastomeric contact

mask in accordance with the principles of the present invention.

Fig. 2 illustrates a process of the invention in which voids are formed using a PDMS contact mask and a substrate.

Fig. 3 illustrates a thermal imprinting process for forming a rigid component for use in one embodiment of the complementary molding process of the present invention.

5   Fig. 4 illustrates a continuous molding process in accordance with the principles of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides patterning elements that are uniquely well suited for patterning biological materials in micro-arrays on a surface.

The present invention further provides methods of forming these patterning elements by a technique dubbed "complementary molding." Complementary molding is a new useful application of soft lithography techniques developed to date.

The patterning elements of the present invention are made from polymers that form gels when contacted with a liquid. A characteristic feature of gel-forming polymers is that they absorb the liquid but do not dissolve in the liquid. Absorption of the liquid often causes a change in the polymer's elasticity, tensile strength and dimensions.

It is generally agreed by those trained in polymer gel technology that gel-forming polymers do not dissolve in liquids with which they form gels because the polymer chains are bound together, such as by covalent bonds as in crosslinking or by extensive physical interactions as in interpenetrating polymer networks. Gel-forming polymers typically are crosslinked polymer networks. Crosslinking between the chains prevents separation of the polymer chains from each other which would lead to dissolution.

Polyelectrolyte gels are also suitable gel-forming polymers of the present invention. Polymers within the class of polyelectrolyte gels possess charges within their network or the ability to stabilize a large number of ions in a polymer network. These polyelectrolyte gels can exhibit electrically induced contraction over time due to electrochemical phenomenon as well as

5   produce electrical potentials due to mechanical deformation. Advantages arising from the use of gel-forming polymers, generally, in the molding process of the invention are discussed below.

Preferred gel-forming polymers of the present invention are hydrogels. A hydrogel is a polymer that absorbs water. Such absorption can be reversible or irreversible. In their hydrated condition, hydrogels are elastically deformable but virtually immune to plastic deformation. In

10   their dry state, hydrogels are often structurally rigid and can be manipulated or formed via known processes, such as machining, etching, and injection molding. Like other gel-forming polymers, hydrogels are typically crosslinked polymers. As used herein, hydrogels can include naturally occurring hydrogels, modified naturally occurring polymers, synthetic hydrogels, and combinations thereof. Hydrogel materials can be charged (e.g. anionic, cationic, ampholytic) or

15   neutral and they can form amorphous and semicrystalline structures. Hydrogel materials include polymers that will absorb water in an aqueous environment and materials that with the addition of either organic or inorganic additives become hydrogel-like by absorbing water.

Exemplary naturally occurring hydrogels include amino acid-based hydrogels, glycoprotein-based hydrogels, carbohydrate-based hydrogels and gums, such as gellan and

20   zanthanes. Exemplary amino acid-based hydrogels include polyalanine and polylysine. Exemplary glycoprotein-based hydrogels include gelatin.

Exemplary carbohydrate-based polymers are dextrins, dextran, dextran sulfate, heparin, heparin sulfate, chitin, chitosan, pullulan, gellan, starch, alginates, hyaluronic acid, agar and

cellulose derivatives such as methylcellulose, hydroxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, cellulose acetate, and sodium carboxymethyl cellulose.

Preferred hydrogels of the present invention are synthetic hydrogels because patterning elements may be readily made from synthetic hydrogels by polymerization of low viscosity mixtures in molds like those described below. The low viscosity of the starting material is advantageous because it enables the faithful reproduction of minute surface features from the molding surface onto the surface of the patterning element.

Synthetic hydrogels may be homopolymers, co- or ter-polymers hydrogels. Synthetic homopolymer hydrogels of the invention are made by polymerizing a hydrophilic monomer or oligomers with a difunctional or multifunctional cross linking agent.

Hydrophilic monomers that can be used to form the hydrogel patterning element include compounds of formula (I):

$$H_2C \overset{\displaystyle \underset{CH_3}{\big|}}{=\!\!C} - \overset{\displaystyle \overset{O}{\|}}{C} - \left(O \diagdown\diagup\right)_n R \quad (I)$$
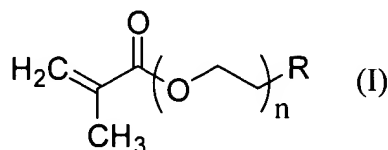
wherein n = 1-12 and R = -H, -CH$_3$, -CH$_2$CH$_3$, -SO$_2^-$, -SO$_3^{2-}$, -CH$_2$NH$_2$, -OH, -OCH$_3$, or -Si(OR')$_3$, and R' is a linear or branched lower alkyl.

Especially preferred methacrylate monomers include 2-hydroxyethylmethacrylate (" HEMA"), methacrylic acid ("MAA"), (1-hydroxypropyl) methacrylate, (2-hydroxypropyl) methacrylate, (2,3-dihydroxypropyl) methacrylate, and polyethylene glycol methacrylates having anywhere from two to about twelve repeat units (*i.e.* n ≤ 12). Other suitable hydrophilic
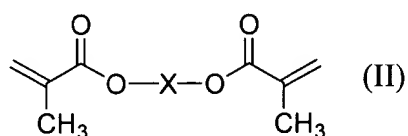
monomers that may be used include acrylates such as acrylic acid ("AA"), 2-hydroxyethyl

acrylate, hydroxypropyl methacrylate, glycerol monoacrylate, methacryloxyethyl glycoside (all

anomers); acrylamides such as diacetone acrylamide, 2-acrylamido-2-methylpropanesulfonic

acid, N-vinyl acrylamide, –methacryloyltris(hydroxymethyl)methylamine; vinyl ester and amide

5   monomers, such as N-vinyl pyrollidinone, –vinyl formamide and vinyl acetate; sulfonyl-

containing monomers such as 3-sulfopropyl methacrylate and vinyl sulfonic acid; polyvinyl

alcohols such as vinyl alcohol and allyl alcohol and hydrophilic ring-opened polymers such as

poly(ethylene imine), and polyepoxide. These monomers may be used individually in a

homopolymerization, in mixtures with each other in a co- or ter- polymerization or in mixtures

10   with other olefinic monomers in a co- or ter- polymerization. In addition, pre-polymer

compositions of oligomers produced from these monomers may be used.

Olefinic monomers may be polymerized by cationic, anionic or radical polymerization

techniques depending upon the particular monomer(s). The above-described hydrophilic olefinic

monomers are conventionally polymerized by radical polymerization. Radical polymerization

15   requires initiation. Initiation may be by radiation, such as UV light, electron beam or high energy

particles from radioactive decay, or by decomposition of a chemical initiator, or both in the case

of a photolabile chemical initiator. Chemical initiators include azo compounds, peroxides,

hydroperoxides and redox systems. Exemplary initiators include lauroyl peroxide, benzoyl

peroxide, isopropyl percarbonate, azobisisobutyronitrile, di-*t*-butyl peroxide, isobutyl peroxy

20   octoate, *t*-butyl peroctoate, *n*-butyl-4-4'-bis(*t*-butylperoxy) valerate and Perkadox®.

Polymerization may be conducted neat or in the presence of solvent.

To produce a crosslinked polymer from mono-olefins, a bis-olefinic or multi-olefinic

crosslinking agent is present. The cross-linking agent binds linear polymer chains together,

thereby preventing dissolution of the polymer, yet allowing penetration of water in the network to modify the physical properties of the polymer. A wide variety of cross-linking agents may be used. One class of suitable cross-linking agents for use with hydrophilic methacrylate monomers may be represented by formula (II):

$$\begin{array}{c}\text{O} \qquad\qquad \text{O} \\ \| \qquad\qquad \| \\ \diagdown\!\diagup\!\!\diagup C\!-\!O\!-\!X\!-\!O\!-\!C\!\diagup\!\!\diagdown \qquad \text{(II)} \\ \text{CH}_3 \qquad\qquad \text{CH}_3 \end{array}$$

where X may be $-(CH_2CH_2O)_mCH_2CH_2-$ where $m = 0\text{-}100$, but more typically 1-25, or $-CH_2C(O)NHCH_2CH_2NHC(O)CH_2-$. The crosslinking agent is typically used in 0.5-10 mole % relative to the total monomer content, more typically about 1 to about 5%.

Other crosslinking agents that may be used include triethanolamine dimethacrylate, triethanolamine trimethacrylate, tris(methacryloyloxymethyl) propane, allyl methacrylate, tartaric acid dimethacrylate, divinyl benzene, N,N'-methylene-bisacrylamide , hexamethylenebis(methacryloyloxyethylene) carbamate, 2-hydroxytrimethylene dimethacrylate, 2,3-dihydroxytetramethylene dimethacrylate, 1,3-butanediol diacrylate, 1,4-butanediol diacrylate, diallyl diglycol carbonate, diethylene glycol vinyl ether, pentaerythritol tetraacrylate, pentaerythritol triacrylate,

Synthetic co-polymer hydrogels of the invention are made by co-polymerizing two monomers, at least one of which is hydrophilic, in the presence of a difunctional or Multifunctional cross-linking agent. Ter-polymer hydrogels are made analogously from three monomers, at least one of which is hydrophilic. The above-described monomers may be co-polymerized with each other. Other exemplary hydrophilic monomers that may be co- or ter-polymerized with the above-described hydrophilic monomers include 2-(N,N-diethylamino)ethyl

methacrylate and hydrolyzed acrylonitrile. Preferred hydrophilic co-monomers for co-polymerization with HEMA include MAA, AA and 2-(N,N-diethylamino)ethyl methacrylate. Co-polymers of HEMA with either MAA or AA can be produced that change dimensions when exposed to aqueous solutions of different pH. Preferred Poly(HEMA-co-MAA) and

5   poly(HEMA-co-AA) hydrogels are preferably made from mixtures containing from about 50% to about 100% HEMA and from about 0% to about 10% MAA or AA.

In a co- or ter-polymer hydrogel, other monomers incorporated into the hydrogel also may be non-hydrophilic. Exemplary non-hydrophilic monomers that may be used include methyl acrylate, butyl acrylate, cyclohexyl acrylate and higher alkyl acrylates; methyl methacrylate,

10   isobutyl methacrylate, n-pentyl methacrylate and higher alkyl acrylates; and α-olefins, such as octene, to name but a few.

Other polymer gel materials that can be made include polyphosphazenes, polyurethanes, polyacrylates, polymethacrylates, poly(ethylene glycol), poly(ethylene glycol) acrylates, 2-(trimethoxysilyloxy)ethyl methacrylate, trimethoxysilyloxyl alkyl methacrylate, trimethoxy silyl

15   alkyl methacrylate, carbohydrate-based hydrogel polymers, polylactic acid, polybutadienes and combinations thereof.

Polymer gels may also be biodegradable. Many biodegradable polymers contain covalent linkages that can be hydrolyzed in biological conditions. Some examples of biodegradable polymer gels are polyhydroxyalkanoates, polylactide, poly(lacticacid-co-aminoacid),

20   polycaprolactone, polyglycolide, poly(lactide-co-glycolide) and combinations thereof. A HEMA, or other hydrophilic polymer, may be made biodegradable by incorporating segments of biodegradable polymer into a block copolymer of HEMA and the biodegradable polymer. The hydrophilic polymer segments may be polymerized as previously described under conditions that

yield low molecular weight polymer chains. The resulting polymer may be polymerized with, for example, lactide, caprolactone or glycolide monomer to form a biodegradable co-polymer. U.S. Patent No. 5,514,380 describes one anionic block copolymerization method that may be used.

The structure of polyHEMA allows for the absorption of water closely approximating that

5   of human tissue. This property, in addition to polyHEMA's resistance to degradation, permeability to metabolites, and relative inertness, make polyHEMA a useful material in patterning biological systems. In view of the foregoing, it will be appreciated that synthetic homo-, co- and ter- polymers that incorporate HEMA monomers are especially preferred hydrogels for making the patterning element of the present invention. Optimal weight average

10   molecular weights of crosslinked polyHEMA hydrogels are from 20,000 to about 2,000,000, more preferably about 100,000 to about 500,000.

One especially preferred polyHEMA hydrogel is formed from a hydrogel precursor composition comprising by weight approximately 97% hydroxyethylmethacrylate ("HEMA"), approximately 2% methacrylic acid and approximately 1% ethyleneglycol dimethacrylate.

15   Another especially preferred polyHEMA hydrogel is formed from a hydrogel precursor comprising by weight approximately 1-10% ethyleneglycol dimethacrylate oligomers where n = 9-12 and approximately 90-99% HEMA.

Patterning elements that may be made from hydrogels using the molding technique and apparatus described below include stamps, such as the PDMS stamps described in U.S. Patents

20   Nos. 5,776,748; 5,512,131 and 5,900,160 and contact masks such as are described in commonly assigned, co-pending U.S. Patent Applications Serial Nos. [atty. docket Nos. 11641/4, 11641/5]. Hydrogels that exhibit faithful elastic memory are well suited to both applications. For instance, a hydrogel stamp is a good patterning element for patterning aqueous solutions of biological

materials onto a substrate. The hydrogels are especially well suited for use as contact masks in the patterning of biological materials upon a substrate and the invention will now be further described with reference to such patterning elements, their production and use.

Contact masks are articles elongated in two dimensions relative to the third dimension.

5   Accordingly, they have expansive bottom and top surfaces compared to the side surfaces. If flexible, their planar shape allows them to conform to, seal with, and mask many surfaces with which they may be contacted. Preferred polymeric gel contact masks for patterning biological materials and chemical materials in bioarrays have a thickness of from about 5 μm to about 3 mm, more preferably from about 5 μm to about 1 mm. Contact masks for patterning biological

10   material also typically have one or more holes extending from the top surface to the bottom surface of the mask. Contacts masks are also useful for protecting the material on a bioarray after deposition and such masks preferably have no holes.

In another embodiment of the inventive polymeric gel patterning elements, the element is a diffusion mask for receiving materials. The diffusion mask, instead of having holes, has

15   cavities that do not extend all the way through the mask. Thus, in such an embodiment, the patterning element is a diffusion mask having a pattern and the biological material is patterned into the cavities of the diffusion mask. The diffusion mask allows selective diffusion of material through the cavities.

Holes or cavities in the contact mask or diffusion mask, respectively, may have any

20   desired shape. Holes and cavities preferably have a cross-sectional area of approximately 1 μm² to several mm². In masking techniques disclosed in commonly-owned co-pending U.S. Application Serial No. [atty. docket No. 1161 1/4], multiple masks are used to pattern multiple biological and chemical materials. In such techniques, the size and shapes of the holes may vary

from one mask to another and holes of various sizes and shapes may be present on the same mask.

The present invention also provides methods of making the polymeric patterning elements using a complementary mold and complementary molding technique. The complementary mold of the present invention includes first and second half molds. One or both of the first and second half molds can be made of an elastomeric material. Alternatively, one of the first or second half molds can be made of a rigid material relative to the other half mold.

Each half mold of the complementary mold has a molding surface. The molding surfaces of the two components define a void between them when the two molding surfaces are placed together in opposition or closed. The molding surfaces can have surface features on them. These surface features have elevated portions and recessed portions. The surface features on the molding surfaces preferably have an aspect ratio of greater than approximately 0.1, more preferably in a range from approximately 0.5 to approximately 5.0 (dimension in the plane of the molding surface over the dimension orthogonal to the plane of the molding surface).

Closing the first and second half molds defines a void between the mold surfaces. The void is formed in the space between the surface features on the first and second half molds. Having at least one elastomeric half mold aids in sealing the half molds. There can be one or more spaces forming the void depending on the surface features in the molding surfaces.

A contact mask may be molded by introducing a molding precursor into the void formed by the first and second half molds and curing it. Molding precursor, as used herein, includes, but is not limited to, prepolymers, polymer precursors, polymer gel precursors, hydrogel precursors, monomers, oligomers, polymers, and crosslinkers. The precursor may be introduced into the void between the molding surfaces before the first and second half molds are placed together in

opposition or closed, or injected into the void after closing the first and second half molds.

Alternatively, a polymer precursor may be introduced to one or both of the mold surfaces before

closing the mold. The molding precursor can be a combination of monomers, oligomers,

polymers, and crosslinkers, which are then polymerized to form the polymer gel contact mask.

5   Alternatively, a solution of polymer can be used. If a solution of polymer is used, the solution

may be heated to evaporate the solvent leaving the solidified polymer.

The meaning of "curing" depends upon the precursor composition. For instance, if the

polymer precursor is a dispersion of naturally occurring or synthetic polymer, curing may be

evaporation of the solvent so as to leave behind a solid or semisolid polymeric gel mass that has

10   conformed to the molding surfaces. In evaporative curing, the solution is typically heated to

accelerate evaporation of the solvent, but that is not strictly necessary.

Naturally occurring hydrogels and modified naturally occurring polymers may be cured into

patterning elements by dissolving in a solvent, introducing the solution into the mold cavity and

evaporating the solvent.

15   Curing of a polymer precursor containing olefinic monomers, oligomers, crosslinking

agents etc. involves polymerization as previously described. Curing of such compositions may

involve heating, but it also may be performed by irradiating the precursor composition. Those

skilled in the art will appreciate that the preferred rigid and elastomeric materials for producing

the mold halves are substantially transparent to visible and near UV light, enabling photo

20   initiation of radical polymerization.

Figure 1 illustrates an embodiment of the complementary molding method of the present

invention using an embodiment of a complementary mold of the present invention to form a

polymer gel contact mask according to the present invention.

As schematically depicted in Figure 1, complementary mold **100** includes a first half

mold **110**, having a square shape, a bearing surface **112** and a molding surface (not shown). In

this embodiment, the first half mold **110** does not have any surface features on its molding

surface. However, first half mold **110** may have surface features. In addition, first half mold **110**

5   can have alignment marks (not shown) on its molding surface in order to properly align it with

the second half mold **130** of the complementary mold **100**. Furthermore, the first half mold **110**

can have a channel (not shown) for discharge of excess molding precursor present during

molding. The first half mold is preferably elastomeric.

The complementary mold **100** also includes a second half mold **130**, which has a bearing

10   surface (not shown) and a molding surface **132**. Second half mold **130** is preferably rigid.

Second half mold **130** may optionally be formed from an elastomer such as PDMS, other

silicones, polyurethanes, polyphosphazenes, natural rubber, and synthetic rubbers such as, latex,

chlorosulorated polyethylene rubber, neoprene, polychlorprene rubber, styrene rubber, nitrile

rubber, and ethylene propylene rubber. Preferably, the second half mold is polyethylene,

15   polypropylene, or Teflon®. The second half mold **130** can be formed by conventional injection

molding or micromachining techniques. A more preferred method of forming the second half

mold **130** is thermal imprinting.

In thermal imprinting as that term is used herein, an impression is made in a workpiece by

thermally softening the workpiece and pressing a master having on its surface a topological

20   reversal of the desired impression against the workpiece. An elastomeric master, and in

particular a PDMS master, can be used to imprint a concave molding surface into a variety of

poly(olefin) and acrylic thermoplastics, including polyethylene, polypropylene, polybutadiene,

polyisoprene and poly(methyl methacrylate). A physical requirement for such imprinting to work

successfully is that the thermoplastic have a lower glass transition temperature, T_g, than the master. Another important property is that the molten thermoplastic have a sufficiently low viscosity that it conforms to the elastomeric master without significantly distorting the master. The necessary viscosity can generally be achieved by adjusting the imprinting temperature, which

5   in no event should be allowed to exceed the T_g of the master. This criteria is particularly important if the molding surface has surface features.

In thermal imprinting, an elastomeric master pattern can be used to transfer surface features to a polymeric article that has a lower glass transition temperature, T_g, than that of the elastomeric master. Thermal imprinting has several advantages: it is inexpensive, many articles

10   may be patterned from a single master, it is a relatively simple process, and releasing the finished substrate is relatively easy.

Figure 3 shows an example of a method of thermal imprinting according to the present invention. The method depicted in Figure 3 can be used to make the components of the mold of Figure 1. In Figure 3, a patterned PDMS master is used to pattern a polyethylene film. Other elastomeric substrates can be used in place of PDMS in performing thermal imprinting, such as

15   those discussed in U.S. Patent No. 5,512,131, which is hereby incorporated herein by reference in its entirety. The embodiment shown in Figure 3 is not limited to molding polyethylene. Both high (T_g~75°C) and low (T_g~50°C) density polyethylene, polymethacrylate (T_g~105°C), polypropylene(T_g~100°C), and any material having a T_g less than the master pattern material

20   could be imprinted using this method as well. Surface features may be imprinted into a molding surface either simultaneously with the imprinting step that forms a mold surface, or in a separate step by applying a second master to the pre-formed molding surface. The surface features on the molding surfaces preferably have an aspect ratio of greater than approximately 0.1, more

preferably in a range from approximately 0.5 to approximately 5.0, to ease release of the cured polymer gel.

Curing of the polymeric gel in the mold cavity forms surface features in the pattern element that are topologically reversed from the features on the mold surface.

5   In one illustrative method of patterning a mold component, the first step of the thermal imprinting process is to place a polyethylene film 310 between a patterned PDMS master 320 and optionally a second PDMS substrate 350. The polyethylene film 310, the PDMS master 320, and optionally the second PDMS substrate 350 are then placed between two rigid plates 330 and 340. Rigid plates 330 and 340 are placed outside the patterned PDMS master 320 and the second

10 PDMS substrate 350. Pressure is then applied to plates 330 and 340 while the temperature of the structure is increased to above the glass transition temperature of the polyethylene 310. As the temperature reaches the glass transition temperature of the polyethylene 310, the polyethylene 310 flows into recesses in the PDMS master 320, forming structures that are topologically reversed replicas of the pattern in the master. Once the polyethylene 310 flows into the PDMS

15 master 320, the structure is cooled to below the glass transition temperature of the polyethylene 310 and the polyethylene 310 hardens. The PDMS master 320 and the second PDMS substrate 350 are then separated from the molded polyethylene 310. The PDMS master 320, and the PDMS master 320 can then be used to thermally imprint another polyethylene film.

In another embodiment of the thermal imprinting process, two patterned PDMS masters

20 can be used to thermally imprint a pattern onto both sides of a polyethylene substrate. The polyethylene substrate that is to be patterned is placed between two PDMS masters such that the patterned surfaces of the PDMS masters contact the polyethylene substrate in a manner like that shown in Figure 3. Two rigid plates are placed on the outside surface of the PDMS masters and

used to apply pressure while the temperature of the structure is raised above the T_g of the

polyethylene. As the temperature is increased, the polyethylene flows into the relief pattern in

both of the PDMS masters, thus forming negative patterns on both surfaces of the polyethylene.

Once the patterns are formed, the temperature is lowered to below the T_g of polyethylene. The

5 PDMS masters are separated from the polyethylene and a polyethylene substrate, patterned on

two sides, is thus formed. Although polyethylene is an exemplary substrate, it would be apparent

to those of skill in the art that other polymers may be patterned using this method.

  Referring back to Figure 1, like the first half mold **110**, the second half mold **130** can

have surface features of any shape. The second half mold **130** may also have a channel **150** for

10 run-off of excess molding precursor during molding. The second half mold **130** also may have

alignment marks to allow proper alignment with the first half mold **110** of the complementary

mold **100**. The alignment marks are especially useful when both the first half mold **110** and the

second half mold **130** have surface features.

  A sealing structure **160** can also be incorporated into either the first or second half mold

15 **110** or **130** respectively. The sealing structure **160** ensures that the molding precursor remains

within the boundaries of the complementary mold **100.** The sealing structure **160** may be an "O-

ring," lip, gasket, or similar structure that is located along or near the edge of either the first half

mold **110** or the second half mold **130.**

  Optionally, a release film **120** may be inserted between the half molds during molding to

20 facilitate release of the polymeric gel patterning element after it has cured. The release layer

isolates the precursor from one of the molding surfaces during molding. Although the release

layer is typically thin, about 10 nm to several hundred microns, the release layer is preferably not

used against a molding surface that has surface features that are to be transferred to the patterning

element. The release layer may be made of a material that is nonadhesive toward the polymeric gel to ease release of the pattern element from the molding surface with which the release layer is in contact. For example, in Figure 1, the release layer **120** can be a thin film of polytetrafluoroethylene or another polymer that has a working temperature above the curing temperature of the polymer precursor and has low interfacial free energy. Such materials include polytetrafluoroethylene, perfluoro silicone, perfluoro polyethylene oxide, polyvinylidene and metal foil (passivated to eliminate adhesion).

Alternatively, the release layer may be a "carrier layer" that is adhesive toward the polymeric gel causing the patterning element to adhere to the carrier layer when the half molds are separated. Such materials include silicone films, metal foils, oxidized polymer films, Mylar® (coated with SiO_2 for example). A carrier layer is advantageously used to separate the polymeric gel from a molding surface that has minute surface features. As will be clear to one of ordinary skill in the art, the carrier layer **120** should be selected according to the properties of the elastomeric contact mask being molded in order to ensure preferential adhesion to the carrier layer **120**.

As an alternative to using a release layer, one or both of the molding surfaces may be coated with a release coating. Nonadhesive coating materials include perfluoro silicones, perofluoro (ethylene oxide) or other non-adhesive material that do not inhibit curing of the polymer precursor. These coatings may be applied by brushing or spraying onto the mold surface.

Additionally, rigid first and second plates **170** and **180**, formed from materials such as aluminum, steel, nickel, glass, or the like, can be used to apply pressure to the first and second half molds **110** and **130** respectively. Pressure is applied by placing the first and second plates on

the bearing surface of the first and second half mold **110** and **130** respectively and applying

pressure to the outside of first and second plates **170** and **180** such that the plates are pushed

together. Pressure may be applied by pneumatic, hydraulic, mechanical or electromechanical

means. Pressures applied may range from 0.1 N/cm^2 to 100 N/cm^2. Applying pressure to the

5   plates thus transmits pressure to the half molds.

   The present invention also provides methods of using the complementary mold. Figure 1

shows an embodiment of how to use complementary mold **100.** First half mold **110** is provided

and a release layer **120** is disposed adjacent to the first half mold **110** along its molding surface.

Release layer **120** may also act as a carrier layer, depending on the polymer contact mask being

10   molded. A second half mold **130** is provided, which has surface structures on a portion of its

molding surface **132.** A void is formed between the molding surfaces of the first and second half

molds **110** and **130** when they are closed. One of the half molds may optionally have a channel

150 for drainage of excess molding precursor and a sealing structure **160** along or near the edge

of its molding surface.

15   A molding precursor may be introduced either before or after closing the mold **100.** If the

molding precursor is introduced into the void before closing the mold **100**, excess precursor will

be squeezed out when pressure is applied through channel **150** if provided. If the molding

precursor is introduced into the void after closing the mold, then the precursor can be injected

into the complementary mold **100**, or it can be allowed to fill the void via capillary forces.

20   Figure 2 shows another embodiment of the mold in which an elastomeric half mold **210**

(e.g. PDMS) is used with a rigid half mold **220** (e.g., polyethylene or glass). A plurality of mold

cavities **230-238** are formed as a result of abutting the contact surfaces of the elastomeric half

210 and the rigid half **220.** Mold cavities **230-238** may be hydraulically separated from one

another, e.g., fluid from mold cavity **230** can be prevented from leaking out of or into mold cavity **232**.

First and second plates **170** and **180** respectively are used to apply pressure to the first half mold **110** and the second half mold **130**. The pressure can range from approximately 0.1

5   N/cm^2 to 100 N/cm^2. In addition, while pressure is being applied the molding precursor is polymerized in a manner appropriate for the polymeric precursor. For example, the precursor may be polymerized using thermal, chemical, electrochemical, or photochemical methods.

The complementary molding process of the present invention may be conducted using lower pressure than typically is used in conventional injection molding and embossing

10   techniques. For example, to accurately complementary mold structures that have features greater than 100 μm, no pressure needs to be applied if the curing temperature is not greater than approximately 60°C depending on the contact mask material. The curing temperature in the case of a polymerization depends in part upon the choice of chemical initiator if one is used. Thus, if one desires to conduct a radical polymerization at less than 60°C one of skill may select a

15   chemical initiator with a half life of about 1 to about 6 hours within the desired temperature range. In addition, the use of a photolabile initiator and irradiation may be adopted.

After polymerizing of the molding precursor is complete, the first half mold **110** and the second half mold **130** are separated to release a new molded contact mask **140**. Release layer **120** may be used to facilitate the separation process.

20   The complementary molding process of the present invention may be automated for mass production. The molding process of the invention may be carried out in a serial, batch, or continuous process. In a serial process, one contact mask is molded per process cycle. In a batch process, more than one contact mask may be molded concurrently, but only those contact masks

are printed during that cycle.

In one such automated process, one or both of the half molds moves relative to a continuously supplied molding precursor. In another automated process, a carrier film can be used to continuously move the molding precursor through the complementary molding process

5 and transport cured polymeric gel away from the mold. In another embodiment of the invention, the complementary half molds and the molding precursor may be moved in concert along a carrier film. Regardless of whether the complementary half molds or the carrier film is moved, the movement may be accomplished by a conveyer belt, reel to reel process, directly-driven moving fixtures or pallets, chain or belt driven fixtures or pallets, a moving cart, a frictional

10 roller, or a rotary apparatus.

According to another automated embodiment of the present invention, the half molds are automatically oriented in a desired or preset configuration relative to each other. The alignment may be accomplished with the use of a sensor or sensors. By way of non-limiting example, these sensors may include charge-coupled devices (for example, a camera), or capacitative, inductive,

15 and displacement sensors. The alignment may also be accomplished with fine positioning actuators. Such positioning actuators may include, for example, micrometer stages, flexural stages, air-bearing stages, magnetic bearing stages, and laser-guided optical alignment.

Figure 4 schematically represents a continuous reel-to-reel complementary molding process, where the carrier film 410 upon which the molding precursor 420 is placed. The

20 complementary mold 430 can be used in the same manner as described above except that the process is repeated as the carrier film 410 moves from the unprocessed reel 440 to the processed reel 450. A first half mold 460 and a second half mold 470, comprising the complementary mold 430, are brought into contact to form a void. The molding precursor 420 fills the void between

the first half mold **460** and the second half mold **470**. As the carrier film **410** moves, the

complementary mold **430** moves as well. Pressure is applied to the complementary mold **430**

and polymerization or solvent evaporation in the molding precursor **420** takes place as the

complementary mold **430** moves toward the processed reel **450**. Before the complementary mold

5   **430** reaches the processed reel, the complementary mold **430** is separated and the cured contact

mask (not shown) is separated from the complementary mold **430**. The contact mask may then

be removed from the carrier film or processed as appropriate for the desired application.

Alternatively, the complementary molding technique may be used with non-film

substrates. For example, a polymer gel membrane may be formed on a pre-cut substrate, which

10   is moved along a conveyor belt.

The invention will now be further illustrated with the following non-limiting examples.

EXAMPLES

Example 1: Molding Using a Mold Having a PDMS Half mold and a Polyethylene Half Mold.


15   PolyHEMA (0.5 ml) precursor containing 97 wt. % of 2-hydroxyethyl-methacrylate

("HEMA"), 2 wt. % of methacrylic acid, 0.5 wt. % of ethyleneglycol dimethacrylate and 0.5 wt.

% of a peroxide free radical initiator, Perkadox® (Kayaku Noury Co.), was introduced into the

void between a PDMS half mold and a polyethylene half mold. The PDMS half mold was flat

without any surface structures. The polyethylene half mold had an ordered array of 100 µm

20   diameter circular posts, 100 µm in height. The mold was placed between two glass plates and

approximately 5-10 N/cm^2 pressure was applied to the bearing surfaces. The mixture was

polymerized by placing the mold in an oven at approximately 85°C for 4 hours. The PDMS and

polyethylene halves were then separated, and the resulting polyHEMA patterned hydrogel contact

mask was removed from the mold.

Example 2: Molding Using a Mold Having a PDMS Half Mold and Polytetrafluoroethylene Half Mold.

5

The molding surface of the PDMS half mold provided surface structures, while the molding surface of the polytetrafluoroethylene half was flat. The patterns on the PDMS half included posts approximately 100 μm in diameter and approximately 50 μm height. The contact mask formed using this complementary mold was made from a polymeric precursor comprising 10% of a mixture of polyethyleneglycol dimethacrylate oligomers:
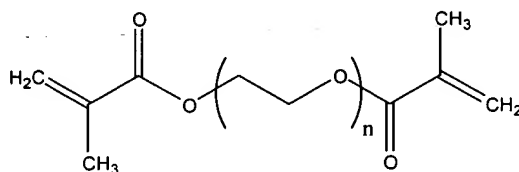
(where n = 9-12), 90% HEMA and 0.1% by weight of the initiator Perkadox®. The polymeric precursor (0.1 ml) was placed on the PDMS master and excess polymeric precursor was allowed to run off as the PDMS and polytetrafluoroethylene half molds were brought together between two glass plates. Approximately 10 N/cm^2 of pressure was applied and the complementary mold was placed in an oven at approximately 85°C for 4 hours. The complementary mold was then cooled to room temperature and the polytetrafluoroethylene half mold was removed. The molded contact mask adhered to the PDMS master, but was removed from the PDMS half mold using transparent tape. The transparent tape peeled off easily after the hydrogel contact mask was hydrated.

Example 3: Use of a Hydrogel Contact Mask to Pattern Biological Materials.

A contact mask as made in Example 1 or 2 was fully hydrated in deionized water for 2 hours at room temperature. The contact mask thus became highly flexible and pliable.

5   The hydrated contact mask was placed on a 5 cm diameter culture dish. The size of the contact mask was approximately 10 mm x 20 mm. The contact mask contained cavities, having 100 μm diameters. After allowing the mask to adhere to the culture dish, the assembly was tilted to drain off excess water and placed in an oven at 37°C for 20 to 40 min to partially dehydrate the hydrogel.

10   When partially dehydrated, the hydrogel contact mask adhered strongly to the culture dish. 5 ml of Dulbecco's Modified Eagle Medium (DMEM) and 10% Calf Serum was added to the membrane and a gentle vacuum was applied to extract air bubbles. The medium was then allowed to hydrate the contact mask.

Freshly trypsinized 3T3 cells (1 ml, 15 cells) were added to the culture dish. The cells were

15   allowed to settle to bottom of the cavities. After 30 min. the unadhered cells were washed off using DMEM/CS medium. Under 5 ml of fresh DMEM/CS medium, the 3T3 cells were allowed to grow. After 24 hours, the hydrogel contact mask maintained a good seal and contact with the substrate. Minimal adhesion of 3T3 cells to the hydrogel contact mask was observed.

20   Example 4: Molding with a Mold Having Two Elastomeric Half Molds with Patterned Mold Surfaces.

Both the first and second elastomeric halves were PDMS and both the first and second

elastomeric halves had surface structures on portions of their molding surfaces. The first and second halves were used in the same complementary mold method as described above, except that in this case both halves forming the sealed enclosure were elastomeric.

The first PDMS half was patterned with posts 5μm in diameter and 10μm in height, and the second PDMS half was patterned with posts 100μm in diameter and 100μm in height.

The polymer formed from this complementary mold was polyurethane. A polyurethane precursor NOA65 (Norland Products) was introduced between the two PDMS half molds, which were placed between two glass plates. Approximately 0.5 N/cm^2 of pressure was applied and the mold was exposed to UV light to polymerize the polyurethane precursor. The two PDMS halves were separated, and the resulting polyurethane contact mask having patterns on both its top and bottom surfaces was removed.

The disclosed embodiments are illustrative of the various ways in which the present invention may be practiced. Other embodiments can be implemented by those skilled in the art without departing from the spirit and scope of the present invention.